Potentiometric Study of Holmium Complexes with EDTMP

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Within the study of $^{166}$Ho-complexes as potential radiopharmaceutical agents, stability constants of those with EDTMP (ethylenediamine-$N,N',N',N'$-tetra(methylenephosphonate)) were calculated by the computer program LETAGROP Etitr using data from potentiometric titrations measured at 25 °C in 0.1 M NaCl. The following values were obtained for stability constants of Ho-EDTMP complexes $\text{Ho(OH)L}_6^-$, $\text{HoL}_5^-$, $\text{HHoL}_4^-$, $\text{H}_2\text{HoL}_3^-$, $\text{H}_3\text{HoL}_2^-$ and $\text{H}_4\text{HoL}^-$: $\log \beta = 9.31, 20.22, 28.14, 34.55, 39.89, 45.05$, respectively. Diagram of the complex speciation dependence on pH shows that complex forms $\text{HHoL}_4^-$ and $\text{H}_2\text{HoL}_3^-$ are characteristic of the pH range 6–8, which is adequate for their application into the human body.

Key words
- holmium
- EDTMP (ethylenediamine-$N,N',N',N'$-tetra(methylenephosphonate))
- potentiometry

EXPERIMENTAL

Chemicals

Solutions of sodium hydroxide of concentrations in the range from 0.01 mol dm$^{-3}$ to 0.09 mol dm$^{-3}$ were used as titration agents. The solutions were prepared from the saturated stock solution of NaOH by the Sörensen method. Titration mixtures were prepared from stock solutions obtained by diluting a requisite amount of substance in an appropriate volume of $5\times10^{-3}$ mol dm$^{-3}$ EDTMP acid and 0.01 mol dm$^{-3}$ holmium nitrate. EDTMP was prepared in the laboratory of the Department of Organic Chemistry, Natural Science Faculty of Charles University in Prague. Holmium nitrate was purchased from Aldrich Prague, Czech Republic. All other chemicals were made at Lachema Brno in the Czech Republic and were of analytical reagent grade purity.

Procedure

Experimental data for the calculation of constants were obtained by potentiometric titration of the required mixtures. An automatic titrator Radiometer RTS 822 with a glass electrode G 2040 and calomel electrode K 4040 was used.
for the measurement of potentiometric data. The glass electrode was calibrated using standard buffer solutions (saturated solution of potassium hydrogen phthalate pH = 4.008, 0.025 mol dm\(^{-3}\) phosphate buffer pH = 6.865, 0.0087 mol dm\(^{-3}\) potassium dihydrogen phosphate / 0.03 mol dm\(^{-3}\) sodium hydrogen phosphate pH = 7.413, 0.01 mol dm\(^{-3}\) sodium tetraborate pH = 9.180, saturated solution of calcium hydroxide pH = 12.454; temperature 25 °C). The accuracy of the calibration procedure was successfully verified by measuring the pH values of the set of acid solutions (standardized by titration). Standard buffer solutions used for the calibration helped extend the applicable pH range of further experiments. Such calibration reduced the deviation of the glass electrode response.

All titrations were performed at a temperature of 25 °C at constant ionic strength of 0.1 mol dm\(^{-3}\) NaCl in the inert atmosphere of nitrogen to avoid CO\(_2\) absorption by the basic solutions. The pH range of the titrations was 2–10. It was found that the kinetics of hydroxide reaction with the ligand was fast enough and therefore the pH values were registered one minute after addition of the titration agent. EDTMP acid was titrated in a concentration range from 5×10\(^{-4}\) mol dm\(^{-3}\) to 2×10\(^{-3}\) mol dm\(^{-3}\). The titrations in the presence of holmium nitrate were carried out at the EDTMP:Ho molar ratio 1:1 and 2:1 for the ligand concentrations 2×10\(^{-3}\) mol dm\(^{-3}\), 1×10\(^{-3}\) mol dm\(^{-3}\) and 5×10\(^{-4}\) mol dm\(^{-3}\). It must be pointed out that no precipitation was formed in the pH region pH = 2–11.

**RESULTS AND DISCUSSION**

The H\(^{+}\)–L\(^{8-}\) system can be characterized by equations (L\(^{8-}\) is the EDTMP anion):

\[ nH^+ + L^{8-} \leftrightarrow H_n L^{(8-n)-} \]  \hspace{1cm} (1)

where Eq. (1) characterizes the protonation of EDTMP, \( n \) is the protonation degree.

Reaction (1) and the following reaction (2) can be expected in the H\(^{+}\)–Ho\(^3+\)–L\(^{8-}\) system:

\[ Ho + nH^+ + L^{8-} \leftrightarrow H_n HoL^{(8-3-n)-} \]  \hspace{1cm} (2)

where Eq. (2) describes the holmium complex formation.

Reactions expressing the protonation of EDTMP and the complex formation allow defining the protonation constant:

\[ \beta = \frac{[H_n L^{(8-n)-}]}{[L^{8-}][H^+]^n} \]  \hspace{1cm} (3)

and the stability constants of complexes:

\[ \beta = (H_n HoL^{(8-3-n)-}) = \frac{[H_n HoL^{(8-3-n)-}]}{[Ho^{n+}][L^{8-}][H^+]^n} \]  \hspace{1cm} (4)

The stability constant of the HoL\(^{5-}\) complex is defined as:

\[ K(HoL^{5-}) = \frac{[HoL^{5-}]}{[Ho^{n+}][L^{8-}]} \]  \hspace{1cm} (5)

and the consecutive protonation constants of H\(_n\)HoL\(^{(8-3-n)-}\) complexes can be described as:

\[ K(H_n HoL^{(8-3-n)-}) = \frac{[H_n HoL^{(8-3-n)-}]}{[H_{n-1} HoL^{(8-4-n)-}][H^+]^{n-1}} \]  \hspace{1cm} (6)

Computer program LTGW Etitr derived from the LETAGROP Etitr model, which was used for the evaluation of experimental data, helps solve the equation system ((1), (2) listed above) and calculate the stability constants defined by Eq. (3) and Eq. (4) by the non-linear regression method.

Protonation constants and complex stability constants were calculated by the program in a number of steps so as to make the calculated pH values approximate the values obtained by experimental measurement. Minimized function \( U \) can be expressed as:

\[ U = \Sigma (pH_{exp} - pH_{calc})^2 \]  \hspace{1cm} (7)

The value of \( pK_w = 13.8 \) was used in the calculation.

Program LTGW Etitr allows visualization of the calculated curve and the experimental values to assess whether the model with the \( U \) function minimum value really fits the experimental data.

Stability constant \( K(HoL^{5-}) \) is identical to \( \beta(HoL^{5-}) \). Consecutive protonation constants can be calculated from the equation:

\[ \log K(H HoL^{(8-3-n)-}) = \log \beta(H HoL^{(8-3-n)-}) - \log \beta(H_{n-1} HoL^{(8-4-n)-}); \quad n > 0 \]  \hspace{1cm} (8)

The data in Table I show the protonation constants of EDTMP acid obtained by the above mentioned procedure.

The estimated protonation constants were used for determination of the stability constants of the Ho(III) complexes. The potentiometric study operates at the metal:ligand molar ratios of 1:1 and 1:2. The accuracy rapidly decreases for higher molar ratios due to the fact that the characters of the titration curve Ho-EDTMP and the titration curve of the ligand without metal are similar. The reciprocal ratios cannot be used because of the metal hydrolysis.

In the calculation, the existence of different protonated (or hydrolyzed) complexes from H\(_n\)HoL\(^{+}\) to Ho(OH)\(_2\)L\(^{7-}\) was assumed. The complex forms Ho(OH)\(_2\)L\(^{7-}\), H\(_2\)HoL and H\(_3\)HoL\(^{+}\) were not proven in the titration solution be-
cause the minimum of $U$ was reached for zero stability constants of these complexes (Figure 1 presents an example of the comparison between the calculated curve and experimental data).

The constants (summarized in Table II) calculated on the basis of the selected model made it possible to demonstrate the pH dependence of the fraction species in the Ho$^{3+}$-EDTMP system (Figure 2). Shares of holmium ions and holmium complexes in the total analytical concentration of holmium are defined as:

$$ \delta = \frac{[\text{H}_i\text{HoL}]}{\sum_{i=1}^n [\text{H}_i\text{HoL}]} \quad (9) $$

The more protonated forms of complexes occur in the acidic pH range and the less protonated forms in the basic range. It can be assumed that the characteristic forms in neutral solutions ($5 < \text{pH} < 8$) are H$_3$HoL$^{2-}$, H$_2$HoL$^{3-}$, and HHoL$^{4+}$. From the value of pH = 6.5, the ratio $\delta$ (Eq. 9) of the completely deprotonated form HoL$^{5-}$ increases, and from the value pH = 8, the Ho(OH)L$^{6-}$ (written as HoH$_4$L$^6-$) ion can be found in the solution. The deprotonated HoL$^{5-}$ form reaches its maximum quantity in the solutions at pH = 9. This complex has a very large range of occurrence (pH~ 6–11). Because of the large number of complexes that can be formed, the ranges of their oc-

### TABLE I. Protonation constants (log β) of EDTMP acid

<table>
<thead>
<tr>
<th>Form</th>
<th>log (β)</th>
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<tbody>
<tr>
<td>HL$^{7-}$</td>
<td>10.3 ± 0.5</td>
</tr>
<tr>
<td>H$_2$L$^{6-}$</td>
<td>20.0 ± 0.4</td>
</tr>
<tr>
<td>H$_3$L$^{5-}$</td>
<td>27.9 ± 0.4</td>
</tr>
<tr>
<td>H$_4$L$^{4-}$</td>
<td>34.4 ± 0.4</td>
</tr>
<tr>
<td>H$_5$L$^{3-}$</td>
<td>39.6 ± 0.4</td>
</tr>
<tr>
<td>H$_6$L$^{2-}$</td>
<td>42.2 ± 0.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temp. / °C</th>
<th>Medium</th>
<th>Lig. conc. / mol dm$^{-3}$</th>
<th>Ref.</th>
<th>This work</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>9</th>
</tr>
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<tbody>
<tr>
<td>25</td>
<td>0.1M NaCl</td>
<td>5×10$^{-4}$, 2×10$^3$</td>
<td>2×10$^{-3}$</td>
<td>1.5×10$^{-3}$</td>
<td>2×10$^{-3}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>0.15M NaCl</td>
<td>2×10$^{-3}$</td>
<td>1.5×10$^{-3}$</td>
<td>2×10$^{-3}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>0.15M NaCl</td>
<td>2×10$^{-3}$</td>
<td>1.5×10$^{-3}$</td>
<td>2×10$^{-3}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>3M KNO$_3$</td>
<td>2×10$^{-3}$</td>
<td>1.5×10$^{-3}$</td>
<td>2×10$^{-3}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>0.1M KNO$_3$</td>
<td>2×10$^{-3}$</td>
<td>1.5×10$^{-3}$</td>
<td>2×10$^{-3}$</td>
<td></td>
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</table>

Note: Standard deviations were calculated at the significance level 3δ.

Figure 1. Titration curves of A) EDTMP; B) Ho-EDTMP molar ratio 1:1; C) Ho-EDTMP molar ratio 1:2. ○ experimental values; – calculated curve.

Figure 2. Fractions of the species containing holmium ion in the system Ho$^{3+}$-EDTMP. The curves were calculated for the constants given in Table I and Table II: 1: Ho$^{3+}$; 2: H$_4$HoL$^{−}$; 3: H$_3$HoL$^{2−}$; 4: H$_2$HoL$^{3−}$; 5: HHoL$^{4−}$; 6: HoL$^{5−}$; 7: Ho(OH)L$^{6−}$.
The dependence of holmium ion and complex shares on pH (Figure 2) are in agreement with the published data. The pH range from 6 to 8 is characteristic of the complex form $\text{H}_2\text{HoL}^{3-}$ and $\text{H}_2\text{HoL}^{5-}$. Concentrations of these forms reach the maximum at pH $\approx 6$ and pH $\approx 7$, respectively. The ion complex $\text{HoL}^{5-}$ could be also detected in the solution under the optimal conditions for radiolabeling.

The values of the stability constants of complexes can be useful in the simulation of their behavior in blood plasma.

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REFERENCES


U okviru ispitivanja kompleksa $^{166}$Ho kao potencijalnih radiofarmaceutika, određene su konstante stabilnosti holmijevih kompleksa s EDTMP (etilendiamin-$N,N',N',N''$-tetra(metilenfosfonatom)) metodom potencijometrijske titracije. Titracije su izvedene kod $25 \, ^{\circ}C$ u $0.1 \, M \, NaCl$, a pri obradi dobivenih rezultata rabljen je LETAGROP Etitr program. Dobivene su sljedeće vrijednosti za konstante stabilnosti Ho-EDTMP kompleksa: $\text{Ho(OH)}L^6^-$, $\text{HoL}^5^-$, $\text{HHoL}^4^-$, $\text{H}_2\text{HoL}^3^-$, $\text{H}_3\text{HoL}^2^-$ i $\text{H}_4\text{HoL}^-$; $\log \beta = 9.31; 20.22; 28.14; 34.55; 39.89$ i $45.05$. Grafički prikaz ovisnosti kompleksa o pH pokazuje da su u području pH između 6 i 8, koje je pogodno za humanu primjenu, prisutne $\text{HHoL}^4^-$ i $\text{H}_2\text{HoL}^3^-$ kompleksne vrste.